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Unmasking intraspecific variation in offspring responses to multiple environmental drivers

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Abstract

Understanding organismal responses to environmental drivers is relevant to predict species capacities to respond to climate change. However, the scarce information available on intraspecific variation in the responses oversimplifies our view of the actual species capacities. We studied intraspecific variation in survival and larval development of a marine coastal invertebrate (shore crab *Carcinus maenas*) in response to two key environmental drivers (temperature and salinity) characterising coastal habitats. On average, survival of early larval stages (up to zoea IV) exhibited an antagonistic response by which negative effects of low salinity were mitigated at increased temperatures. Such response would be adaptive for species inhabiting coastal regions of freshwater influence under summer conditions and moderate warming. Average responses of developmental time were also antagonistic and may be categorised as a form of thermal mitigation of osmotic stress. The capacity for thermal mitigation of low salinity stress varied among larvae produced by different females. For survival in particular, deviations did not only consist of variations in the magnitude of the mitigation effect; instead, the range of responses varied from strong effects to no effects of salinity across the thermal range tested. Quantifying intraspecific variation of such capacity is a critical step in understanding responses to climate change: it points towards either an important potential for selection or a critical role of environmental change, operating in the parental environment and leading to stress responses in larvae.

Keywords: *Carcinus maenas*, global invaders, life cycles, marine larvae, multiple stressors.

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Introduction

Climate change is leading to a multiple modification of the physical and chemical properties of Earth habitats towards conditions that have not been experienced in the recent past (Gattuso and Hansson 2009; IPCC 2014; Gunderson et al. 2016; Boyd et al. 2018). Climate change affects multiple environmental variables that are key drivers of physiological and ecological processes (Brierley and Kingsford 2009; Hoegh-Guldberg and Bruno 2010; Doney et al. 2012; Sokolova et al. 2012, Torres et al. 2019). Whether such changes lead to positive or negative effects depends on species, communities or ecosystems, and hence they are difficult to predict. However, there is an urgent need to increase the capacity to predict how organisms will respond to such changes if we are to be able to mitigate the effects of climate change on ecosystem services and goods.

Biological responses to multiple environmental variables or drivers cannot be predicted from the isolated effects of each driver (also termed "stressors": Folt et al. 1999; Crain et al. 2008; Piggott et al. 2015; but we follow the logic of Boyd et al. 2018 in that the effects of a driver can be also positive). For instance, several reviews (Crain et al. 2008; Harvey et al. 2013; Kroeker et al. 2013; Côté et al. 2016; Gunderson et al. 2016) have found a widespread occurrence of synergistic or antagonistic responses at the level of individuals (e.g. survival, growth or development rates) to the community and ecosystem levels (e.g. species diversity, primary production). Synergistic and antagonistic responses are stronger or weaker, respectively, than those expected from the action of each single environmental driver (see e.g. Folt et al. 1999; Crain et al. 2008; Piggott et al. 2015 for definitions) and hence cannot be predicted from studies focusing on single drivers. Because such interactive effects are widespread and represent a major source of uncertainty, there is currently an important level of research effort focusing on understanding their nature. Characterising the nature of the responses is important for developing strategies to mitigate the effects of human activities on populations or ecosystems (Côté et al. 2016; Schäfer and Piggott 2018).

At the organismic level, an important source of uncertainty concerns intraspecific variation in the responses to multiple environmental variables, because most studies focus on inter- rather than intra-specific variations (but see e.g. Carter et al. 2013, Durrant et al. 2013). However, responses can vary within a species (and possibly within a population) due to parental effects (Marshall et al. 2008; Uller et al. 2013; Parker et al. 2017) and genetic variation (Nasrolahi et al. 2012; Durrant et al. 2013; Appelbaum et al. 2014), or perhaps due to both sources (Carter et al. 2013). Parental effects, i.e. the effects of the parental environment on offspring performance, are expected to occur in response to variations in maternal nutrition (Cowgill et al. 1984; Pond et al. 1996) or parental temperature (Donelson et al. 2011; Shama et al. 2014). Both sources of variation can affect, for instance, offspring

size or body mass, which in turn can drive offspring performance (Giménez and Anger 2003; Marshall et al. 2008).

Intraspecific variation can have important ecological consequences (Bolnick et al. 2011). Most notably, if intraspecific variation in responses to environmental drivers is high, average trends do not truly represent the magnitude of the species response to the drivers especially when such traits contribute non-linearly to fitness, a phenomenon known as the Jensen inequality (Denny 2017). Another important point is that an average lack of effect of an environmental driver can potentially mask both positive and negative effects on the performance of individuals or lineages (Appelbaum et al. 2014). Hence, studies addressing the magnitude of intraspecific variation in multiple driver responses will potentially unmask the existence of phenotypes that thrive under environmental change; they can unmask potential adaptive eco-evolutionary dynamics or portfolio effects (Bolnick et al. 2011; Schindler et al. 2015) that will be relevant to species persistence. In that sense, low levels of variation (due to genetic heterogeneity) would compromise population persistence and would require specific conservation strategies targeting (at least) the offspring habitat. On the other hand, variation that is non-adaptive, driven by a suboptimal maternal environment (e.g. see Parker et al. 2017), will indicate the need for conservation strategies targeting (at least) the maternal habitat. The focus on intraspecific variation provides the stepping-stone towards understanding how trait variation drives responses to climate change.

Here, we quantify intraspecific variation in multiple driver responses of larvae of the shore crab *Carcinus maenas* to temperature and salinity. *C. maenas*, is native to Europe, but it is also considered a global invader elsewhere (Roman and Palumbi 2004; Compton et al. 2010). *C. maenas* develop through four zoeal stages and a megalopa settling on shore habitats (Spitzner et al. 2019); larvae occur in coastal waters and semi enclosed seas, where they are exposed to variations in temperature and salinity. Particularly marginal and semi-enclosed seas currently experience an important influence of climate change (Philippart et al. 2011; Robins et al. 2015). We study a population located in the German Bight (North Sea), that has been exposed to increases in temperature experienced over the past decades (Wiltshire et al. 2010; Meyer et al. 2011), which in addition may undergo a further increase of 1-3°C by 2100 (Schrum et al. 2016). We focus on salinity as a second driver because regional changes in salinity are expected in response to climate change (Gunderson et al. 2016). Shore crabs, as other coastal organisms, will necessarily have to deal with natural variations of salinity in the new scenario of increased temperature, where increases in metabolic demands may not be necessarily met by resources supply. From that perspective, climate change exposes coastal organisms to conditions not previously experienced for many generations. We focus on larvae because larval stages of marine invertebrates are often the most sensitive stage to multiple drivers

(Przeslawski et al. 2015, Pandori and Sorte 2019) as their tolerance spectrum is often narrower compared to their adults (Pechenik 1987; Charmantier 1998). Larvae determine gene flow and population connectivity (Palumbi 2003; Cowen and Sponaugle 2009). Although there are studies investigating the effect of temperature and salinity on *C. maenas* larvae (Dawirs 1985; Nagaraj 1993; Anger et al. 1998), there is very limited information about the magnitude of intraspecific variation in the response to these drivers.

We quantified the magnitude of intraspecific variation in the survival and duration of development in larvae hatching from broods carried by ten different females collected over two years. As first step, we report the average responses and then the variation from the average. For survival, we tested both additive and multiplicative null models of responses. For duration of development, we tested additive and multiplicative models, and evaluated responses with reference to predictions made by models used in metabolic theories (O'Connor et al. 2007). By using such models, we expected to contribute towards a mechanistic approach to study developmental responses of larvae to multiple environmental drivers; such approach is needed for a better understanding of effects of climate change on organisms, as much as for communities or ecosystems (De Laender 2018).

Material and Methods

Animal husbandry, larval rearing and elemental analysis

Carcinus maenas berried females were collected on the island of Helgoland (North Sea, German Bight, Latitude: 54.1771903, Longitude: 7.884409) on two consecutive years (May to August: 2016 and 2017). Larvae in the German Bight commonly experience temperatures of 15 and 18°C during spring and summer (Wiltshire et al. 2010). However, these temperatures are likely to increase in the future due to both steady increase in temperatures (1-3°C for end of century, Schrum et al. 2016) and increase in the frequency of warm years (Christidis et al. 2015). Salinities in the German Bight oscillate in the range of 20-33, depending on distance to the Elbe and Wesser Rivers (see e.g. Bils et al. 2012). Females whose embryos were at a late stage of embryonic development were transported to the laboratory (Helgoland, Germany). They were kept individually in 2-L aquaria filled with natural filtered (0.2-µm) seawater at 18°C and fed with shrimps (*Crangon crangon*) which are the optimal conditions for ovigerous females of this species. Water was changed daily to ensure high water quality at hatching. To avoid confounding effects of acclimation to the laboratory conditions, only larvae that hatched within 48 hs. of collection of the female were used.

Zoeae I hatched from each female were distributed in 12 treatments (4 replicates per treatment; each replicate consisted of 50 larvae cultured in 400-mL glass bowls). Treatments comprised a

factorial combination of four temperatures (15, 18, 21 and 24°C) and three salinities (20, 25 and 32 = seawater) with the temperature 15°C and the natural seawater (salinity 32) as the control conditions. Temperatures below 20°C are considered within the range that may be experienced in nature while those above 20°C represent treatments of thermal stress; osmotic stress is expected with salinities of 25 and 20.

Temperatures were controlled by running experiments in temperature controlled rooms (range ±0.5°C); salinity (range ±0.1 salinity) was controlled using a salinometer (WTW). Experiments were run using natural seawater; waters of lower salinities were obtained by diluting natural seawater with appropriate amounts of tapwater. Daily, larvae were fed *ad libitum* with *Artemia sp*. and water was changed. During the daily water change, larvae were monitored for moults and dead larvae were recorded and discarded. We repeated the experiment five times each year, using five females per year. In both years, larval rearing was carried out by the same team, in order to minimise variation in larval responses due to different people manipulating larvae from different females.

We estimated body mass, carbon and nitrogen content in freshly hatched larvae in order to explore if body mass and nutritional reserves at the initiation of the larval phase would explain intraspecific variations in response to temperature and salinity. Previous studies (e.g. Giménez & Anger 2003) have found positive correlations between reserves at hatching and survival and duration of development. Five replicate samples of larvae hatched of each female (50 freshly hatched Zoea I each) were used to determine elemental Carbon and Nitrogen (details in Torres et al. 2016). Larvae were quickly rinsed with distilled water, blotted dry with filter paper, placed in pre-weighted Aluminium cartridges and stored at -20ºC for subsequent analysis. To determine the dry mass (DW), all samples were freeze-dried for 48h. (Christ Alpha 1–4 freeze-drier) and then weighed on a microbalance (Sartorius SC2, nearest 0.0001-mg). Carbon and Nitrogen content were then determined using an elemental Analyser (vario MICRO cube CHNS analyser, Elementar Analysensysteme).

Data analysis

Cumulative survival until each zoeal stage was calculated as the percentage of survivors with reference to the initial number of freshly hatched larvae (i.e. at the start of the experiment). Cumulative duration of development until each stage was calculated as the time needed to reach the next developmental stage including developmental duration of previous stages. The combined effects of temperature and salinity, as well as intraspecific variations in the responses were evaluated through mixed modelling (Zuur et al. 2009; Galecki and Burzykowski 2013) by using the “lme” function from

the "nlme" package (Pinheiro et al. 2018) in R thought RStudio (RStudio Team 2018). The analyses were carried out in two steps: first, the random terms (i.e. the factor female with its interactions) were tested using restricted maximum likelihood (REML) fitting. Models with different random structure were compared through the Akaike information criteria (AIC). Models were ranked according to their AIC. The model with the lowest AIC score was selected for further analysis. When further analysis was not possible with the chosen model (with lowest AIC score), the second lowest AIC ranked model was used. In the second step, the fixed terms (all terms not containing the factor female) were estimated by maximum likelihood (ML). Tukey's HSD (Honestly Significant Difference) posthoc test was used to determine differences among treatment combinations. Tests for survival were performed after re-scaling the proportions using the equation p' = [p (50-1)+0.5]/50 in order to avoid inconsistencies with proportions =0.

We first evaluated the overall, larval responses using temperature and salinity as fixed factors, and female of origin as random factors (crossed with the fixed factors). The full model contained estimates of variance by combinations of female of origin, temperature and salinity but did not contain co-variances between these terms; using the *lme* function, the random part of the model was coded as *"random= list(ffem = pdDiag(~fsal*ftemp))"*, where *fsal*, *ftemp* and *ffem* denote salinity, temperature and female of origin as factors. Alternative models contained random terms depending on the levels of fixed factors (e.g. as *"random= 1+ fsal|ffem"* or *"random= 1+ ftemp|ffem"*) or only random intercepts associated to the female of origin (e.g. as *"random= 1|ffem"*). The best models corresponded to the full model, i.e. retaining random effects and indicating environmental dependent maternal influences on larval performance (see results).

For survival, we used logarithmic and logistic data transformations prior to the analysis. The logarithmic transformation was used in order to meet the requirements to test the independent (=multiplicative) effect of temperature and salinity on survival probabilities (i.e. an additive model in the logarithmic scale would correspond to a multiplicative model in the scale defined by survival probabilities), but its resulting residuals deviating considerably from the normal distribution (evaluated as qq-normal plots). The logistic transformation by contrast gave residuals with little deviations from the normal distribution. Overall, both approaches retained the same factors in the best models.

For duration of development we run analyses in the raw and log-transformed scales in order to determine whether effects were additive, multiplicative (=additive in the log-scale) or interactive in both scales. In addition, we evaluated the thermal dependence of duration of development with reference to the so-called "universal temperature dependence" model (UTD: O'Connor et al. 2007, their equation 3 and Fig. 3). The thermal dependence of metabolism predicts an inverse relationship

between temperature and developmental duration. Importantly, the UTD enables to test underpinnings of the combined responses to temperature and salinity as it is derived from a mechanistic model linking biochemical level processes and whole organisms metabolic rates. The UTD predicts that duration of development should follow a pattern described by the Arrhenius function, $A(T) = a \cdot e^{f}$ *with* $f=b/[k(T+273)]$, (T is temperature in degrees Celsius; *a* is a constant depending on the body mass, *b* is the "activation energy" (measured in electron Volts, eV), and k=8.62×10^{-5} is the Boltzmann constant). O'Connor et al. (2007) fitted the Arrhenius function, to duration of development of marine larvae of 69 species and found: $a = exp(-22.47)$, $b = 0.64$ *eV*. For the UTD, we log transformed the data of duration of development in order to use linear statistical models to determine if the thermal response followed the Arrhenius function. Under such transformation, we obtain $\log(D) = c_0 + c_1 \cdot f$. (*with* c_0 the intercept and c_1 the slope) as the null model; we refer to f as the "Arrhenius transform" (*f* included $b=0.64$). If the logarithm of the duration of development were linear with respect to *f*, irrespective of salinity, then we retained the Arrhenius function as the best model explaining the thermal dependence of duration of development. In that case, effect of salinity should only appear in the intercept or the slope. Effects on only the intercept should manifest as parallel curves differing in the value of c_0; this would mean that the intercept, predicted to vary with body mass (term *a* fitted by O'Connor et al. 2007), varies also with salinity. Effects on the slope (c_1) would mean that the activation energy depends on the salinity. The alternative option is that the Arrhenius function does not predict effects of temperature on duration of development and in that case, the response should be non-linear. Here, we used a quadratic function as an alternative model: $\log(D) = c_0 + c_1 \cdot f + c_2 \cdot f^2$. The linear and quadratic models were evaluated with polynomial regression, using the orthogonal polynomial approach for tests and the raw polynomial approach for the estimations of parameters. In both cases, models were run with two interacting covariates (salinity and *f*) and random terms defined by the combination of the factor "female" and the covariates. Because initial inspections of data (see Fig. 2 in Results) suggested that duration of development was linear in *f* at the control salinity (=32), we introduced salinity in the models as a new covariate, $StS = 32 - S$, i.e. standardizing each value of the salinity (S) to that of the control. Hence, the fixed component of the full model was: $\log(D) \sim StS + f + StS:f + f^2 + StS:f^2$. If the Arrhenius function captures the functional response of development time, then such model would be reduced to: $\log(D) \sim StS + f + StS:f$ or some simpler model containing *f* (e.g. $\log(D) \sim StS + f$). If the response were not consistent in any salinity, the best model would be $\log(D) \sim StS + f + StS:f + f^2$. If salinity drives the deviations from the Arrhenius function the best model would contain the quadratic term $StS:f^2$.

The role of initial larval nutritional reserves (body mass, Carbon and Nitrogen content) as predictor of survival and duration of development was evaluated through general least square models. First, survival and development data (four replicates per female) were averaged for each female and salinity-temperature combination; larval traits at hatching (three replicates per female) were also averaged and used as predictor variables. Separate analyses were run for dry mass, Carbon and Nitrogen per individual and percent of Carbon and Nitrogen. In each analysis, the full model contained, in the fixed structure the full factorial interaction (*fsal:ftemp:trait*) and the variance model included a correlation structure to control for repeated measures (corCompSymm constructor function) and variance heterogeneity (VarIdent constructor function). Model selection was carried out using the corrected Akaike information criterion (AICc) due to low number of replicates (n=10 for each treatment combination). Best models were represented using the package *effects* in R, which enables to construct scatterplots of partial effects of covariates and interaction terms.

Results

In order to describe intraspecific variation, we start with the quantification of the average responses and then compare variations among females with reference to the average responses.

Average responses

Best models evaluating cumulative survival rates included the interactive effect of temperature and salinity for all tested larval stages (Table 1). The average response consisted of an antagonistic effect whereby increased temperatures (especially at 21, but also at 24°C) mitigated the negative effects of low salinity on survival (Fig. 1a-c). One can appreciate the magnitude of the mitigating effect by comparing the observed survival under the combination of low salinity and high temperature with that expected under independent effects of these conditions. For example, the average survival up to the Zoea II at the control (temperature =15°C; salinity =32) was 0.74 and decreased to 0.34 at the same temperature but at the lowest salinity tested (Fig. 1a). At temperatures as high as 21 and 24°C, survival at the lowest salinity (20) were 0.56 and 0.50; these values were more than two times larger than the expected survival under the independent effects of temperature and salinity (expected for 21°C: 0.23 = 0.69 x 0.34; expected for 24°C: 0.24 = 0.70 x 0.34). At salinity 25, survival was similar to that observed in seawater. The mitigation effect was strong in survival to stage II at both 21 and 24°C, while it was only present at 21°C in survival to stages III (Fig. 1b) and IV (Fig. 1c).

Salinity and temperature affected the duration of zoeal development in opposite directions, with shortened development at high temperatures and lengthened development at low salinity (Fig. 1d-f). Best models for duration of development retained the salinity:temperature interaction term (Table 1).

These interactive effects were antagonistic, especially in the raw scale (Fig. 1 d-f) whereby the effect of low salinity in increasing duration of development was mitigated at high temperatures. For example, at 15°C, the effect of the lowest salinity (20) was to extend by 5.5 days the duration of development to Zoea II (with reference to the control salinity =32), while at 24°C it was extended only by two days (Fig. 1d). Similar responses were observed by comparing duration of development at salinity 25 *vs.* 32, i.e. clear effect of salinity at 15°C but rather similar values at 24°C. An antagonistic response was found also for the duration of development to stages III (Fig. 1e) and IV (Fig. 1f), i.e. with stronger effects of low salinity at 15°C than at 21 or 24°C. Duration of development at salinity 25 did not differ from that of larvae reared in seawater except in larvae reared at 15°C. Interactive effects of temperature and salinity were also found in the logarithmic scale, but the effect was weaker; as compared to sea water, low salinity (20) extended development by 1.43-1.50 times at 15°C vs 1.20-1.34 times at 21-24°C. Overall, responses were not consistent either with an additive nor with a multiplicative model, although deviations from the latter were not large.

Duration of development to Zoea II and IV responded non-linearly to the Arrhenius function (Fig. 2; Table 2). In general, the strength of the non-linear relationship increased towards the lower salinities as captured by the quadratic term (Table 2). Overall, in agreement with the patterns observed in Figure 2, models predicted that reduced salinity would lead to a stronger deviation from the linear relationship between duration of development and the Arrhenius function.

Intraspecific variability

The analysis of interactive survival responses by female of origin revealed three main patterns (Fig. 3, top panels). First, in larvae from five females (females 1, 2, 5, 8 and 10) there were antagonistic patterns (in agreement with the general response), albeit of different magnitude (Fig. 3: compare salinity 20 *vs*. 32); for instance, the effect of low salinity on survival to Zoea II was much stronger at 15-18°C than at 21-24°C (see also Fig. S1 for subsequent stages). Second, in other two females (3 and 6), patterns differed qualitatively from the antagonistic response. In larvae produced by female 3, there was no effect of salinity (two-way ANOVA $p > 0.05$ for interaction term and salinity). In those produced by female 6, there was a multiplicative effect (two-way ANOVA, non-significant interaction but significant effect of salinity and temperature: both $p < 0.001$) meaning that the cumulative effect of temperature and salinity was explained as the product of the effect of each factor in isolation. Third, there was an important overall variation in larval survival (e.g. compare females 8-9 *vs.* females 1-6) as well as variation in the temperature at which survival peaked in larvae

reared at the lowest salinity (at 15-18°C in females 6-7; at 21-24°C in females 1-5). The patterns observed for survival to the second stage were also present for survival to stages III and IV (Fig. S2).

Interactive responses of duration of development were in general consistent with the average antagonistic pattern, whereby the effect of low salinity in extending development was mitigated at high temperatures (Fig. 3, bottom panels: exceptions: females 6 and 9: effects were additive). The predominance of antagonistic responses was also observed in the duration of development stages III and IV (Fig. S1). Such response was particularly strong in larvae produced by females 1 and 2 (Figs. 3 and S1) where, in addition, we observed the strongest deviation from the linear responses when development was plotted with respect to the Arrhenius transform (Fig. S3). Exceptions were found in larvae from females 6 and 9, where the pattern was synergistic (duration of development increased towards higher temperatures in larvae reared at the lowest test salinity). In larvae from these two females, the Arrhenius plot showed a rather linear response of development to temperature at low salinity (Fig. S2).

We used correlation analysis to explore relationships between larval performance at different temperature-salinity combinations; such correlations may reflect the nature of integration among traits that are relevant to stress tolerance (e.g. physiological compensatory mechanisms). Correlations of survival were positive, but variable (Fig. 4, Table S1). Correlations were high ($r > 0.7$) and significant among treatments characterized by salinities 25 and 32 or at high temperatures but they decayed towards salinity 20 and low temperatures (15 and 18°C). Overall, larval survival at the control condition (temperature = 15°C, salinity = 32) was not a good predictor of survival under the highest temperature and the lowest salinity (Fig. 4, $r < 0.62$, n.s. for all stages); hence, survival responses under the putative "multiple stressor" (temperature = 24°C, salinity = 20) treatment were not well predicted from those of the control. For duration of development, correlations were positive and high (Table S1); there was only a decay for specific treatment combinations. Duration of development under control conditions was a good predictor of that exhibited by larvae reared at the putative multiple stressor treatment for Zoea II and III ($r>0.75$, $p<0.05$), but not for Zoea IV (Table S1).

Relationships between survival and larval reserves at hatching were not significant for any indicator of larval nutritional reserves, stage or temperature-salinity combination. Relationships between duration of development and larval reserves at hatching were weak (Fig. S3 and S4), contingent on the salinity and present only for the 3rd and 4th zoeal stage only when percent Carbon (%C) was used as descriptor of larval reserves. Best model for development to Zoea III retained %C:salinity:temperature) and Zoea IV (%C:salinity): in both cases, increases in percent Carbon led to a decrease in duration of development, in larvae reared at the lowest salinity treatment.

Discussion

Here we addressed the issue of intraspecific variation responses of larvae of the shore crab *Carcinus maenas* to key coastal environmental drivers (temperature and salinity). We first characterised the average responses and then examined deviations from the average; through such approach, we found an important level of intraspecific variation in the survival and duration of development. On average, we found an antagonistic response (both in survival and duration of development) that we call "thermal mitigation of low salinity stress", because negative effects of low salinity (lower survival or extended development) were mitigated by high temperature. The thermal mitigation of low salinity stress may be considered a form of cross-tolerance (Fregly 2011) consistent with that described for other coastal species (Kinne 1971; Anger 1991; Janas and Spicer 2008; González-Ortegón and Giménez 2014). Mechanistically, it might result from the fact that compensatory physiological mechanisms controlling osmoregulation are enhanced at high temperatures (Flügel 1963; Campbell and Jones 1989; Janas and Spicer 2008) through an increase in the capacity of mitochondria to produce ATP (Pörtner 2010). Extracellular osmoregulation for instance, is driven by pumping Na^+ by the Na^+-K^+-ATPase located in the ionocytes; intracellular regulation may also be more efficient at higher temperatures. Overall, antagonistic responses have important ecological relevance at the species to ecosystem levels (Côté et al. 2016; Lange and Marshall 2017). For example, the form of thermal mitigation studied here implies that a temperature increase may lead to temporary niche expansion, assuming that such increase does not change other critical environmental factors. Hence, under such scenario increased temperature may favour range expansion by improving larval performance in general (deRivera et al. 2007) and also providing zoeal stages with additional suitable habitats, characterised by moderately low salinity (but >20).

For duration of development the response was also antagonistic especially in the raw scale. Our best fit was a quadratic model based on the Arrhenius function, where the importance of the quadratic term increased because of responses at the combination of low salinities and temperatures. O'Connor et al. (2007) found that responses of duration of development to temperature, in larvae of a number of marine organisms, would fit better a quadratic model (albeit different in structure from ours), but they also found consistent fit of the UTD at temperatures > 7°C. Explaining the non-linearity found by us might require the consideration of additional effects of low salinity, on e.g. body mass (not considered here.

We expected to find that intraspecific variation would consist on slight deviations of the average patterns. We did so for duration of development; however, for survival, clear antagonistic responses were restricted to larvae originating from five females; some showed either no effects or high sensitivity to low salinity. Such responses may reflect genetic variation as well as parental effects.

Moksnes et al. (2014) also reported important variation in larval behavioural traits in the same region than our study; they attributed such variation to gene flow from the northern North Sea. However, for gene flow to explain increased tolerance to low salinity, our local population would need to be connected to those influenced by the Baltic Sea; models (Moksnes et al. 2014) as well as genetic data (Roman and Palumbi 2004; Domingues et al. 2010) speak against this hypothesis. Instead, the observed variation may be explained through important gene flow with populations from NW European Seas (Roman and Palumbi 2004). Alternatively, the observed variation might originate in fluctuations in the temperature and salinity experienced by parents or embryos (Laughlin & French 1989, Giménez and Anger 2001; González-Ortegón and Giménez 2014). Such a mechanism may point towards potential population bottlenecks, caused by a suboptimal maternal environment. Overall, the large magnitude of intraspecific variation found here points toward the necessity to find the underlying causes.

Through correlation analysis, we attempted to find some indications as to which traits or processes may explain the observed levels of intraspecific variation. First, we reasoned that if variation in the same set of traits was responsible for the variation in performance at all temperature-salinity combinations, we would expect high correlations in performance among such conditions; in addition trade-offs may be reflected in negative correlations in physiological tolerance to opposite extreme conditions or to extreme conditions in different environmental variables. We found that survival was highly and positively correlated across temperatures in larvae reared in seawater and at salinity 25 suggesting that performance at those conditions is based on a shared set of physiological traits. We also found that correlations were low for survival of larvae reared at 20 *vs.* other salinities, suggesting that the traits driving tolerance to low salinity differed from those driving survival at other conditions. Second, we tested if variation in larval reserves at hatching would predict variation in survival and development. Following theory (Kindsvater and Otto 2014) and previous results (Giménez and Anger 2003; González-Ortegón and Giménez 2014), we expected that larger offspring size or biomass would result in better performance (i.e. higher survival rates and shorter duration of development) but we found no such evidence for survival and only weak evidence for duration of development. Correlations between duration of development and nutritional reserves were significant only at the lowest salinity and for percent Carbon. Although such pattern would be consistent with the hypothesis that different set of traits govern performance at low *vs.* moderate-high salinities, such relationships were weak. Intraspecific variation in performance may be driven either by concomitant variation in traits that are relevant to stressor tolerance such as those driving physiological repair mechanisms or osmoregulation (Lucu and Towle 2003; Cieluch et al. 2004).

Overall, our data lead us to the following main conclusions and hypothesis. First, that it is important to be aware of potentially intraspecific variation in response of organisms to climate driven environmental factors; as implied in Appelbaum et al. (2014) the average response will not tell the whole picture. Correlation analysis suggest that traits driving variation in tolerance to low salinity are not the same as those driving variation in survival at high salinities. Based on previous studies (Giménez and Anger 2003, G. Torres unpubl. data for *C. maenas* larvae) we hypothesise that environmental conditions experienced by embryos are a likely driver of some of the observed variations, although we do not discard other sources. Understanding such sources is a priority to predict the likely responses to climate change: variability originated in genetic diversity might lead to a form of storage effect (Bolnick et al. 2011) through selection and local adaptation to future thermal conditions. However, the same variability, when driven by a suboptimal maternal environment (e.g. unfavourable temperatures) might lead to population decline.

Authors' contributions: LG, SH and GT conceived the experiments. FS, RM and GT performed the experiments. FS and LG analysed the data. FS wrote the first draft as part of her doctoral dissertation. LG and GT wrote the final manuscript. All authors improved the final manuscript.

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Conflicts of interest: The authors declare that they have no conflicts of interests.

Compliance with Ethical Standards: The research presented in this paper complies with the guidelines from the directives 2010/63/EU of the European parliament and of the Council of 22nd September 2010 on the protection of animals used for scientific purposes.

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639

Figure Captions

Figure 1. *Carcinus maenas*. Effects of temperature and salinity on average survival and duration of development from hatching to the Zoea IV. Cumulative survival to Zoea II (a), Zoea III (b) and Zoea IV (c); cumulative duration of development to Zoea II (e), Zoea III (f) and Zoea IV (g). Bars indicate standard errors among larvae produced by different females (n=10 for zoeal survival and development).

Figure 2. *Carcinus maenas*. Relationships between average duration of development (from hatching to each zoeal stage) and temperature, plotted according to the Arrhenius transform (*f*), for larvae reared at different salinities. Bars indicate standard errors among larvae produced by different females (n=10).

Figure 3. *Carcinus maenas*. Variability in the effects of temperature and salinity on average survival (top panels) and duration of development (bottom panels) from hatching to the Zoea II. Each panel depicts responses observed in larvae produced by a single female (numbered from 1 to 10). Bars indicate standard errors among replicate groups of larvae produced by each separate female (n=4). Symbols as in Figure 1. Notice for instance the differences in the survival patterns between larvae from female 1 (antagonistic), 3 (no effect) and 9 (overall low larval survival). Data corresponding to subsequent stages are given in Figure S2.

Figure 4. *Carcinus maenas*. Surface plot of correlations between average survival proportions in larvae reared in seawater (32) and at 15°C *vs.* those reared at other combinations of temperature and salinity. The average survival proportion was estimated from hatching to moulting to stages II, III and IV in larvae produced by 10 females reared at 12 salinity-temperature combinations. Surfaces were computed as a bi-cubic spline smooth. The full correlation matrix is given in Table S1.

663 **Tables**

664

665 Table 1. *Carcinus maenas*. Summary of model selection (AIC scores) for mixed models evaluating
666 the effect of temperature and salinity on cumulative survival and duration of development of larvae
667 from hatching to Zoea II, III and IV. Models with lowest AIC were retained; model selection was
668 carried out through Restricted maximum likelihood fitting (REML) for the random structure and with
669 maximum likelihood for the fixed structure (ML).

670

	Survival						Duration of development					
Scale:	Logistic			Logarithmic			Raw			Logarithmic		
Random	ZII	ZIII	ZIV	ZII	ZIII	ZIV	ZII	ZIII	ZIV	ZII	ZIII	ZIV
F:S:T (full)	1231	1220	1227	709	801	916	1331	1627	1854	-665	-828	-890
F:T	1369	1379	1402	836	989	1091	1401	1691	1918	-540	-741	-778
F:S	1321	1336	1336	843	960	1044	1350	1674	1909	-608	-785	-802
F	1424	1431	1434	933	1054	1133	1428	1731	1970	-518	-721	-728
Fixed terms												
T:S	1222	1211	1218	685	781	899	1322	1630	1860	-725	-892	-957
T+S	1258	1241	1247	726	816	923	1391	1682	1926	-709	-882	-940

671

672

673 Table 2. *Carcinus maenas*. Parameter estimates and significance of polynomial regression
674 explaining the effect of temperature and salinity through the universal temperature dependence of
675 metabolic rates (UTD). Temperature is included in the UTD through the Arrhenius equation with
676 known parameters, which here is contained in the term *f*. Salinity (StS) is expressed with respect to
677 the control (StS = 0 for larvae reared under control salinity). Parameter estimates correspond to the
678 polynomial fitting in the raw form; significance (* $p < 0.05$, ns: non-significant) was evaluated using
679 the orthogonal polynomial approach. The models fitted at each salinity are given at the bottom of the
680 table by setting the non-significant parameters to zero. Notice that under control conditions, StS = 0,
681 all terms containing StS vanish; for other salinities, the linear terms are recalculated from the
682 parameter estimates, with $f = 0.64/[8.62 \cdot 10^{-5} \cdot (T+273)]$.

683

Random	*Zoea II*		*Zoea III*		*Zoea IV*	
Intercept	0.2316		0.1715		0.1424	
StS	0.0083		0.0054		0.0064	
Residual	0.1236		0.1004		0.0099	
Fixed	Estimate	SE	Estimate	SE	Estimate	SE
Intercept	242.10	88.44	352.70	44.45	232.72	71.01
f	-19.51	6.97	-28.22	3.50	-18.77	5.59
StS	24.10	11.12	-0.14	0.08	22.98	9.10
f^2	0.40	0.14	0.57	0.07	0.38	0.11
f:StS	-1.91	0.88	0.01	0.0003	-1.81	0.72
f^2:StS	0.04	0.02			0.04	0.01
Control	$Ln(D)=242-20\ f+0.40\ f^2$		$Ln(D)=353-28.2\ f+0.57\ f^2$		$Ln(D) = 233-18.8\ f+0.38\ f^2$	
Salinity 25	$Ln(D)=410-33\ f+0.66\ f^2$		$Ln(D)=352-28.2\ f+0.57\ f^2$		$Ln(D) = 394-31.5\ f+0.63\ f^2$	
Salinity 20	$Ln(D)=531-42\ f+0.85\ f^2$		$Ln(D)=351-28.1\ f+0.57\ f^2$		$Ln(D) = 508-40.5\ f+0.81\ f^2$	

684

685

Figures

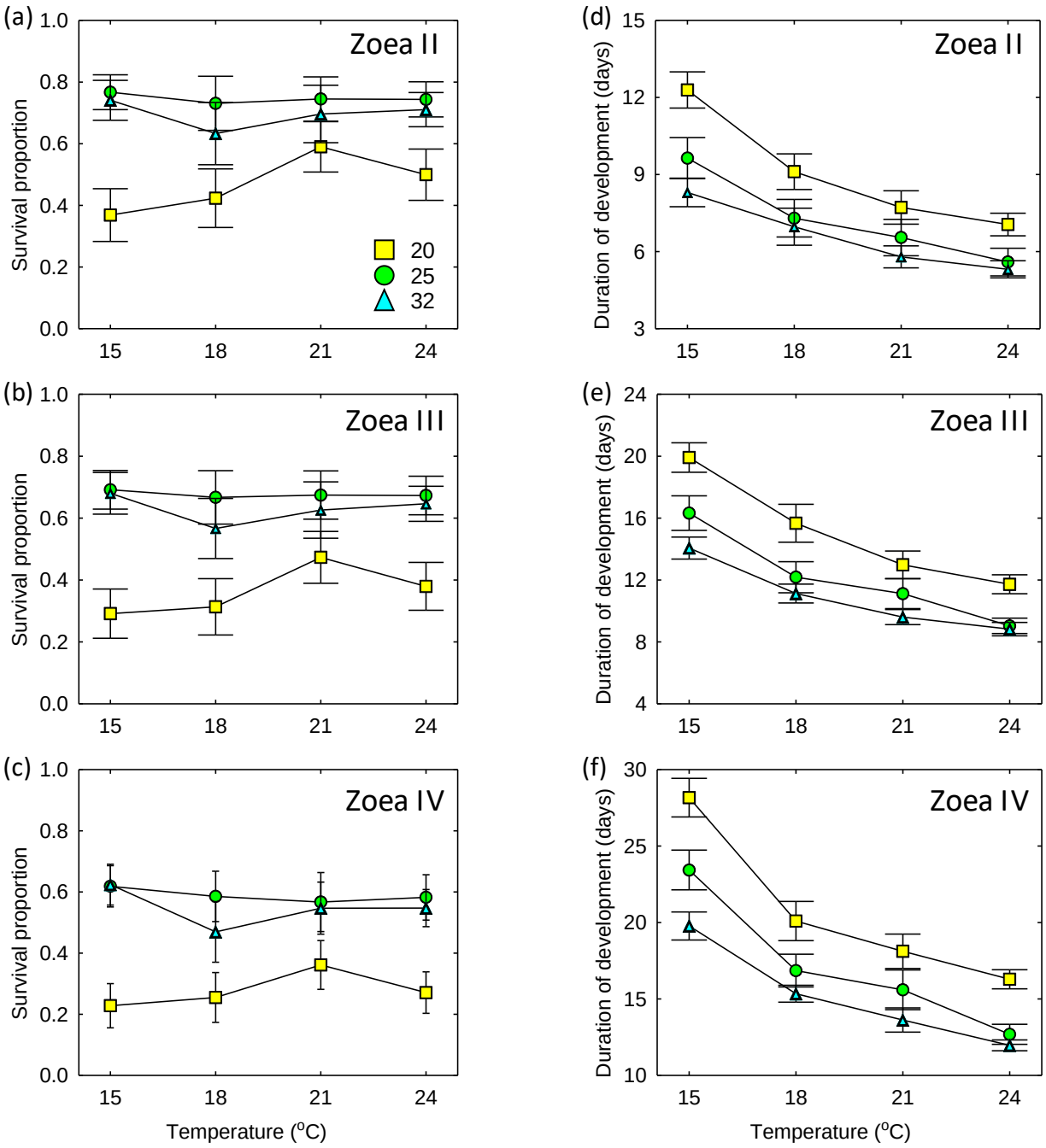

Figure 1

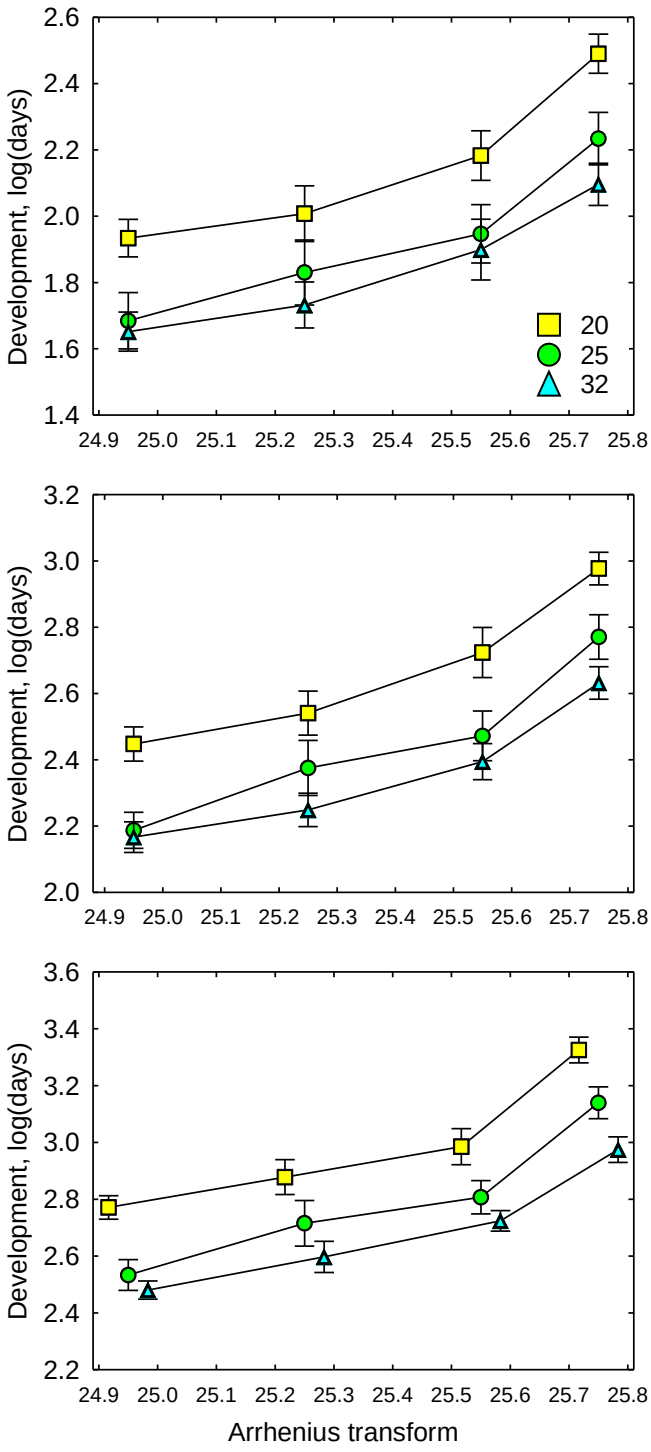
Development, log(days)
2.6
2.4
2.2
2.0
1.8
1.6
1.4
20
25
32
24.9 25.0 25.1 25.2 25.3 25.4 25.5 25.6 25.7 25.8
Development, log(days)
3.2
3.0
2.8
2.6
2.4
2.2
2.0
24.9 25.0 25.1 25.2 25.3 25.4 25.5 25.6 25.7 25.8
Development, log(days)
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
24.9 25.0 25.1 25.2 25.3 25.4 25.5 25.6 25.7 25.8
Arrhenius transform

Figure 2

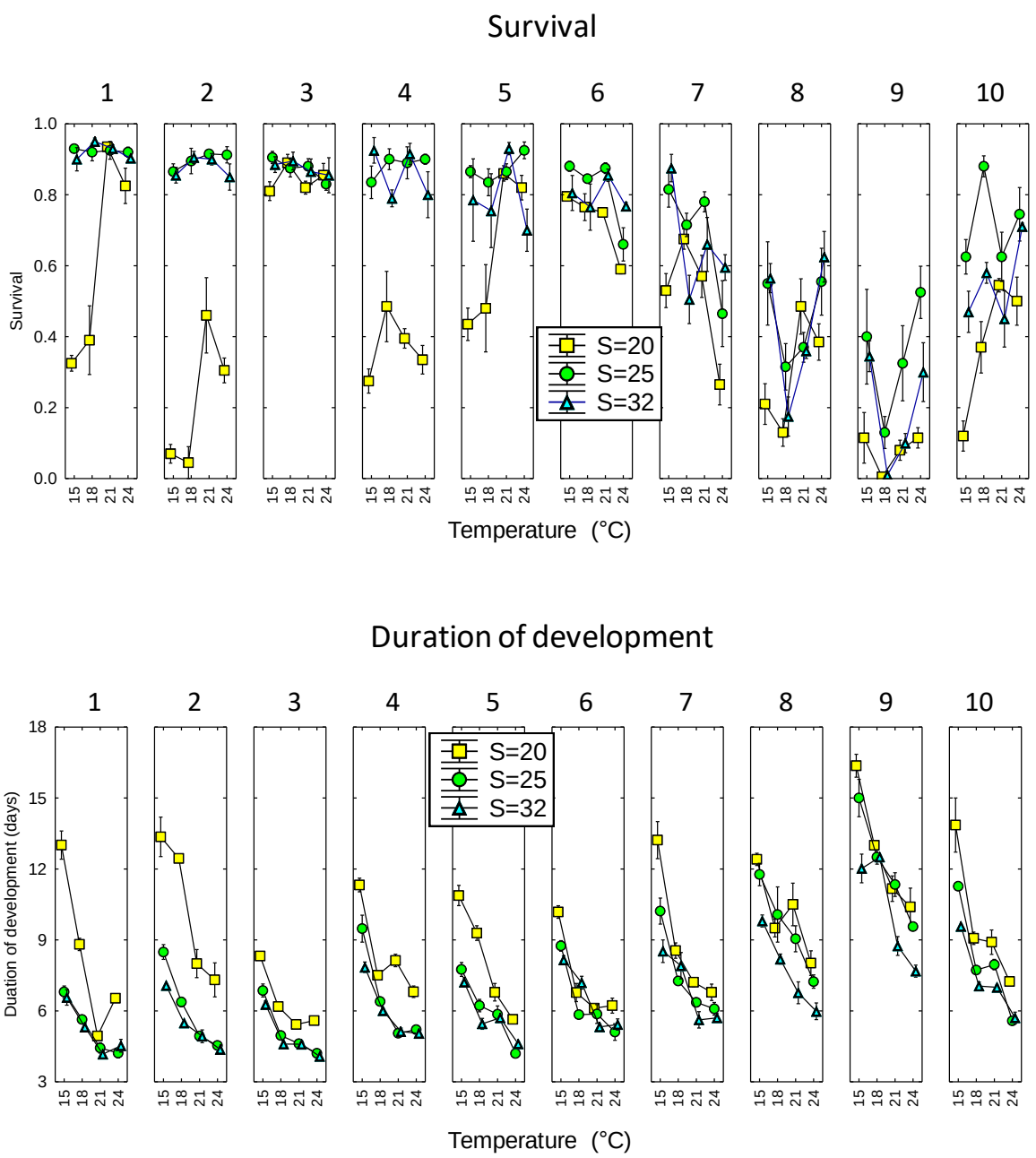
Survival
1
2
3
4
5
6
7
8
9
10
Survival
1.0
0.8
0.6
0.4
0.2
0.0
15
18
21
24
S=20
S=25
S=32
Temperature (°C)
Duration of development
Duation of development (days)
18
15
12
9
6
3
Temperature (°C)

Figure 3

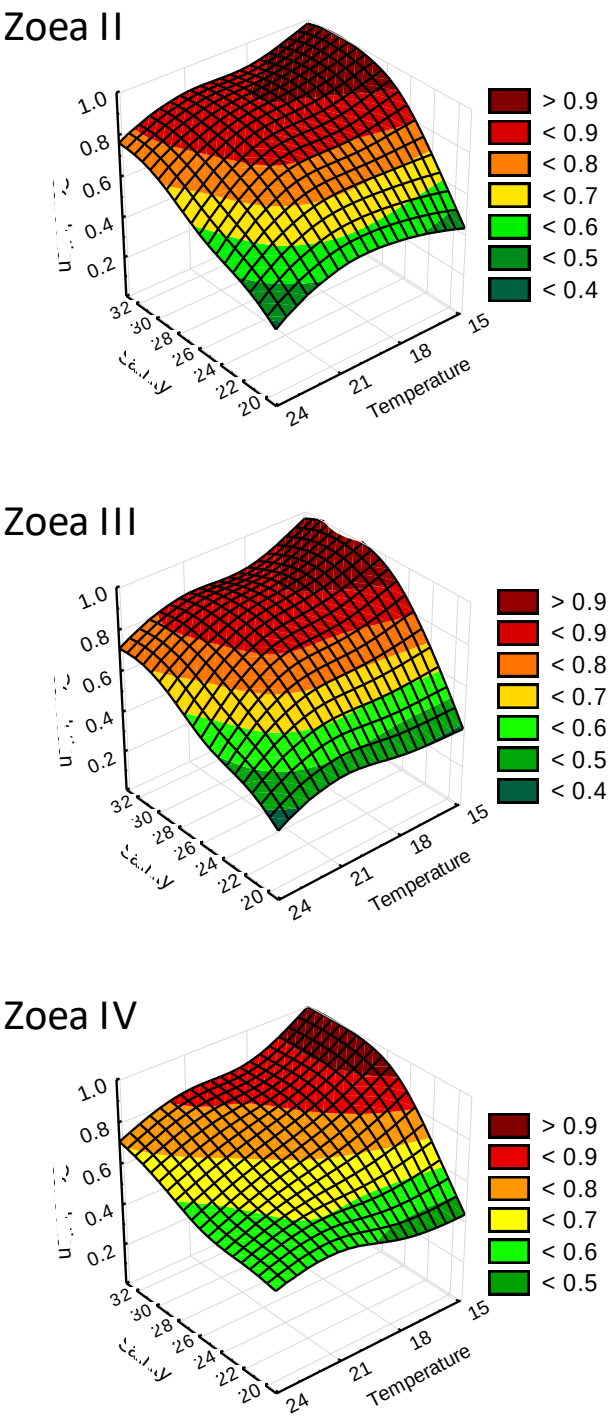
Zoea II
1.0
0.8
0.6
0.4
0.2
32
30
28
26
24
22
20
24
21
18
15
Temperature
> 0.9
< 0.9
< 0.8
< 0.7
< 0.6
< 0.5
< 0.4
Zoea III
1.0
0.8
0.6
0.4
0.2
32
30
28
26
24
22
20
24
21
18
15
Temperature
> 0.9
< 0.9
< 0.8
< 0.7
< 0.6
< 0.5
< 0.4
Zoea IV
1.0
0.8
0.6
0.4
0.2
32
30
28
26
24
22
20
24
21
18
15
Temperature
> 0.9
< 0.9
< 0.8
< 0.7
< 0.6
< 0.5

Figure 4

Supplementary material

Supplementary figures

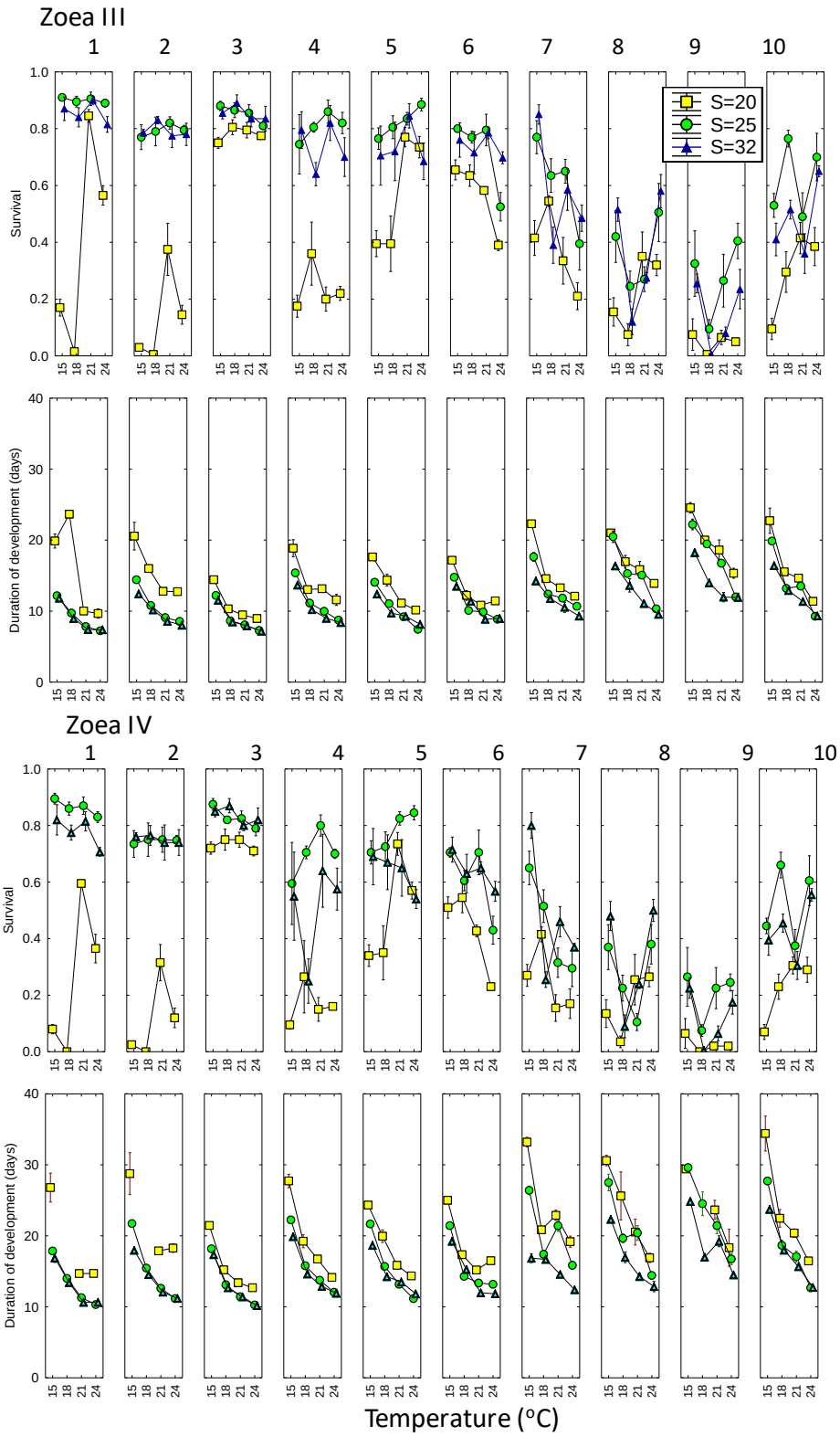

Figure S1. *Carcinus maenas*. Variability in the effects of temperature and salinity (S) on average survival and duration of development from hatching to the Zoea III and Zoea IV. Each panel depicts responses observed in larvae produced by a single female (numbered from 1 to 10). Bars indicate standard errors among replicate groups of larvae produced by each separate female (n=4).

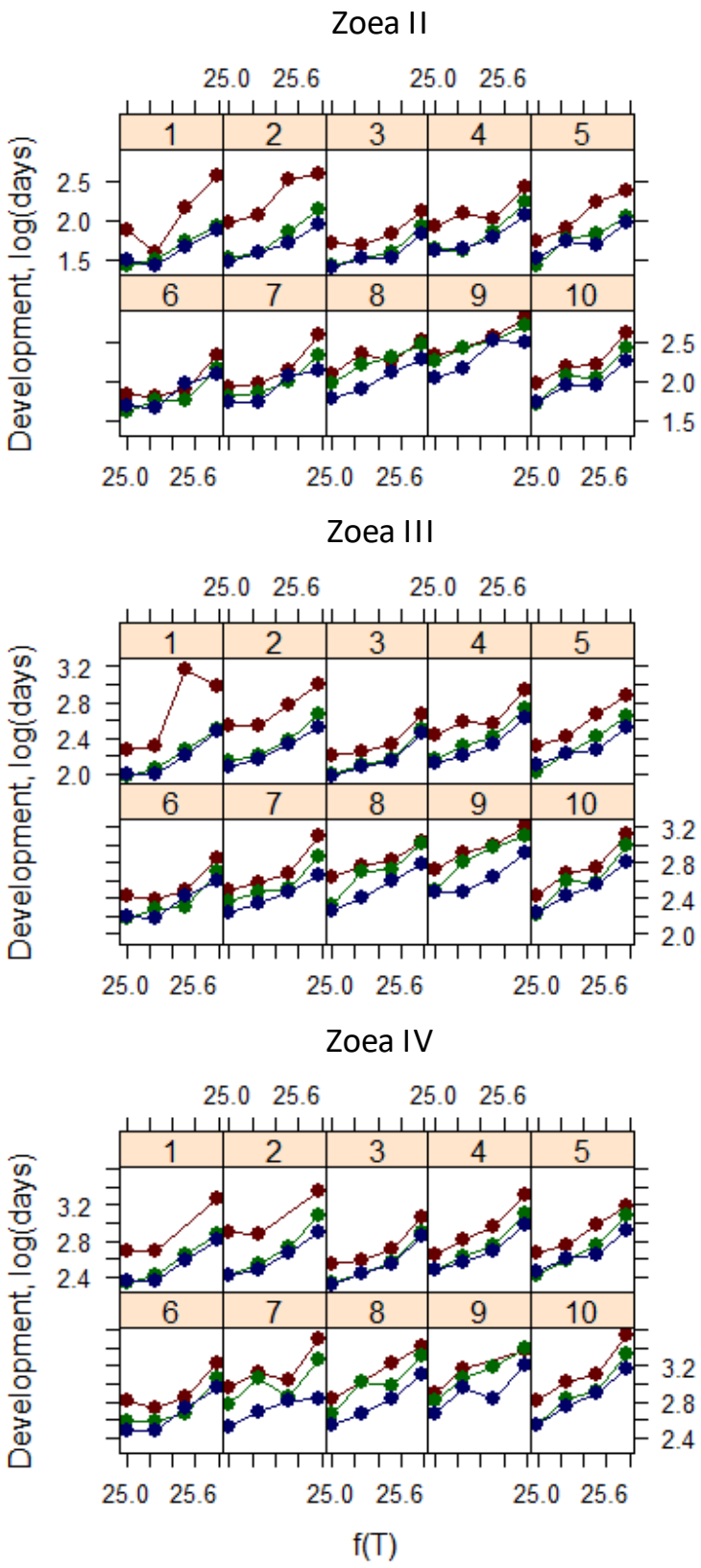

Figure S2. *Carcinus maenas*. Variability in the relationship between average duration of development and temperature plotted in the Arrhenius transform, f(T), for larvae produced by 10 females (numbered from 1 to 10) and reared at three salinities. Symbols of different colours refer to different salinities as follows: red = 20, green =25, blue =32.

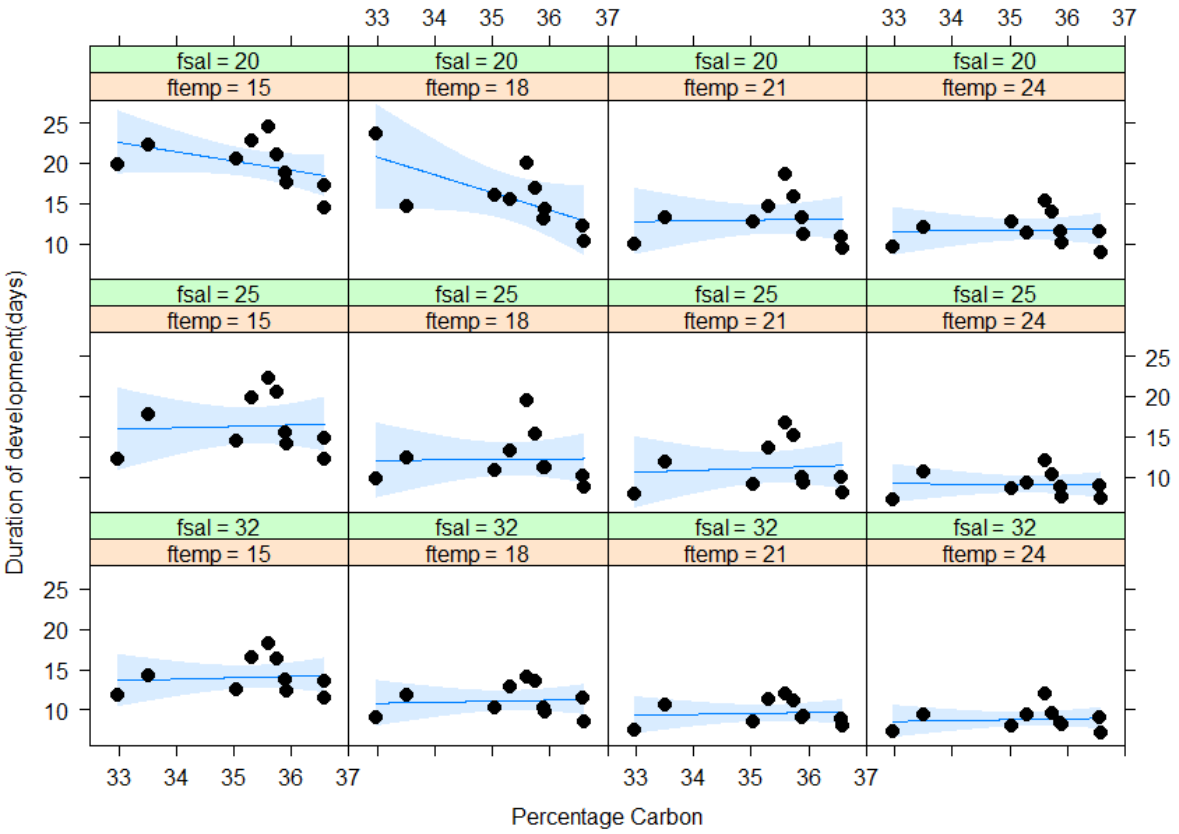

Figure S3. *Carcinus maenas*. Model fit for the relationship between average duration of larval development to Zoea III and percent Carbon at hatching, in larvae produced by 10 females and reared at twelve combinations of salinity (fsal) and temperature (ftemp). Black circles represent the partial residuals and the blue areas represent the confidence bands.

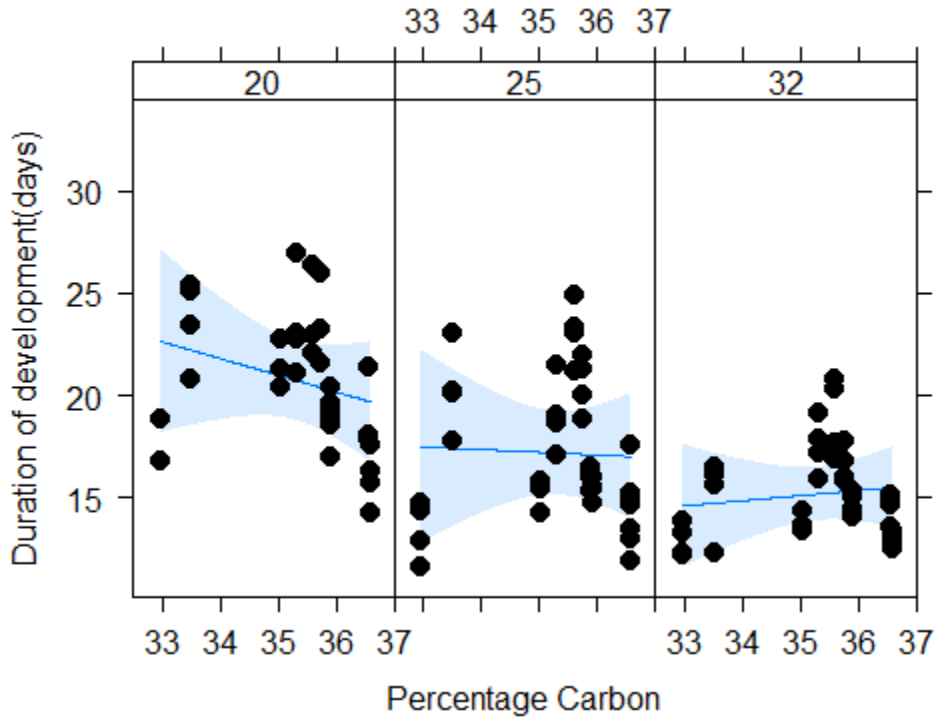

Figure S4. *Carcinus maenas*. Model fit for the relationship between average duration of larval development to Zoea IV and percent Carbon at hatching, in larvae produced by 10 females reared at three salinities (20, 25 and 32). Black circles represent the partial residuals and the blue areas represent the confidence bands.

Supplementary table

Table S1. *Carcinus maenas*. Correlation matrix for larval performance (survival: upper sector; duration of development: lower sector) to stages II, III and IV among larvae produced by 10 different females reared at 12 combinations of salinities (S) and temperatures (T). Significant correlations are in red. For duration of development to Zoea IV, the number of replicate units was 7 due to increased mortality at some temperature salinity combinations.

Zoea II

S	→	20				25				32			
↓	T↓→	15	18	21	24	15	18	21	24	15	18	21	24
20	15	1.00	0.92	0.61	0.55	0.56	0.33	0.46	-0.04	0.51	0.38	0.45	0.30
	18	0.83	1.00	0.64	0.57	0.64	0.55	0.58	0.08	0.58	0.50	0.54	0.42
	21	0.67	0.64	1.00	0.92	0.78	0.67	0.67	0.48	0.61	0.71	0.72	0.71
	24	0.84	0.76	0.85	1.00	0.63	0.58	0.55	0.59	0.43	0.65	0.61	0.64
25	15	0.74	0.57	0.92	0.91	1.00	0.88	0.97	0.63	0.94	0.94	0.98	0.85
	18	0.75	0.66	0.91	0.94	0.95	1.00	0.92	0.72	0.74	0.95	0.87	0.88
	21	0.67	0.55	0.87	0.86	0.95	0.97	1.00	0.70	0.90	0.96	0.97	0.83
	24	0.69	0.54	0.85	0.92	0.96	0.97	0.94	1.00	0.52	0.81	0.75	0.76
32	15	0.72	0.52	0.88	0.88	0.99	0.95	0.97	0.95	1.00	0.83	0.92	0.77
	18	0.72	0.55	0.77	0.90	0.94	0.93	0.92	0.97	0.95	1.00	0.94	0.92
	21	0.67	0.56	0.87	0.83	0.95	0.93	0.98	0.90	0.96	0.89	1.00	0.86
	24	0.71	0.48	0.79	0.87	0.96	0.92	0.94	0.96	0.98	0.99	0.92	1.00

Zoea III

S	→	20				25				32			
↓	T↓→	15	18	21	24	15	18	21	24	15	18	21	24
20	15	1.00	0.91	0.55	0.61	0.53	0.36	0.42	-0.01	0.49	0.40	0.45	0.31
	18	0.60	1.00	0.36	0.49	0.48	0.44	0.40	0.00	0.44	0.38	0.40	0.28
	21	0.84	0.32	1.00	0.92	0.71	0.67	0.63	0.61	0.57	0.73	0.69	0.73
	24	0.76	0.30	0.92	1.00	0.55	0.56	0.50	0.61	0.41	0.60	0.56	0.63
25	15	0.81	0.23	0.95	0.84	1.00	0.89	0.96	0.60	0.95	0.91	0.96	0.79
	18	0.80	0.41	0.96	0.88	0.93	1.00	0.91	0.75	0.76	0.95	0.89	0.86
	21	0.77	0.26	0.95	0.85	0.99	0.95	1.00	0.72	0.87	0.94	0.98	0.79
	24	0.79	0.20	0.90	0.90	0.91	0.89	0.91	1.00	0.47	0.79	0.72	0.80
32	15	0.78	0.26	0.94	0.82	0.99	0.93	0.99	0.89	1.00	0.78	0.91	0.72
	18	0.77	0.22	0.90	0.85	0.97	0.88	0.97	0.90	0.96	1.00	0.93	0.91
	21	0.76	0.12	0.90	0.76	0.98	0.89	0.96	0.87	0.95	0.94	1.00	0.83
	24	0.76	0.26	0.91	0.87	0.92	0.95	0.93	0.93	0.93	0.91	0.89	1.00

Zoea IV

S	→	20				25				32			
↓	T↓→	15	18	21	24	15	18	21	24	15	18	21	24
20	15	1.00	0.92	0.61	0.70	0.48	0.28	0.33	0.13	0.51	0.46	0.41	0.34
	18	0.82	1.00	0.47	0.60	0.44	0.37	0.33	0.12	0.47	0.38	0.38	0.29
	21	0.72	0.78	1.00	0.92	0.75	0.69	0.68	0.75	0.64	0.84	0.70	0.70
	24	0.78	0.57	0.82	1.00	0.58	0.55	0.49	0.63	0.51	0.64	0.52	0.60
25	15	0.59	0.91	0.94	0.69	1.00	0.88	0.85	0.71	0.94	0.88	0.96	0.79
	18	0.72	0.98	0.88	0.59	0.91	1.00	0.87	0.86	0.74	0.87	0.91	0.84
	21	0.59	0.80	0.95	0.71	0.93	0.85	1.00	0.85	0.66	0.82	0.92	0.73
	24	0.46	0.60	0.89	0.75	0.87	0.82	0.94	1.00	0.51	0.79	0.79	0.81

32	15	0.86	0.68	0.59	0.28	0.79	0.84	0.58	0.55	1.00	0.76	0.88	0.69
	18	0.55	0.81	0.87	0.70	0.95	0.78	0.86	0.79	0.74	1.00	0.87	0.85
	21	0.60	0.82	0.86	0.54	0.91	0.96	0.82	0.81	0.81	0.78	1.00	0.84
	24	1.00	0.89	0.86	0.62	0.93	0.95	0.86	0.88	0.83	0.85	0.94	1.00

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